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13. ABSTRACT (Maximum 200 Words)

Elevated serum levels of insulin-like growth factor 1 (IGF1) have been found in prostate cancer patients, and IGF1-related signal transduction is thought to be an important factor in the development of prostate cancers. The goals of this project are to discover small organic molecules that suppress IGF-activated prostate cancers by cell-based screening and to analyze their action mechanisms. In the first year of funding, we discovered, from our collection of synthetic compounds, the drug-like compound we call 125B11 that suppress IGF1-dependent growth of prostate cancer cells but not serum-dependent growth. During this period of funding, we analyzed the mechanism of action of 125B11 to gain molecular insights into how IGF1 stimulates the growth of prostate cancer cells. DNA microarray and cell biological experiments indicated that 125B11 modulates the function of sterol regulatory element binding protein (SREBP), a transcription factor that activates specific genes involved in cholesterol synthesis, endocytosis of low density lipoproteins, and the synthesis of both saturated and unsaturated fatty acids. Chemical genetics of 125B11 may reveal a novel crosstalk between fatty acid metabolism and prostate cancer progression.

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Introduction

Elevated serum levels of insulin-like growth factor 1 (IGF1) have been found in prostate cancer patients, and IGF1-related signal transduction is thought to be an important factor in the development of prostate cancers (*1*). The goals of this project are to discover small organic molecules that suppress IGF-activated prostate cancers by cell-based screening and to analyze their action mechanisms. To discover such molecules from a chemical library, we took a unique two-step approach: we first examined the phenotypic effects of chemical library members (10,000 divergent drug-like compounds) on the insulin-induced adipogenic differentiation of cultured fibroblasts, and then identified, from the pool of the non-cytotoxic compounds that blocked the insulin-induced adipogenesis, organic compounds that suppress IGF-mediated growth of prostate cancer cells. In the first year of funding, we successfully discovered 125B11 through the two-step approach. The chemical genetic analysis of 125B11 may reveal new insights into how IGF1 stimulates the growth of malignant prostate cancer cells.

Body

In the second year of funding, we focused on Task 2:

To analyze mechanism of action of 125B11

In the first year of funding, we discovered 125B11, a compound that suppress IGF1-dependent growth of prostate cancer cells. The drug-like thiazole derivative specifically inhibited the IGF1-induced growth of DU-145 cells at IC50 of 0.1 μM but had little effects on their serum-induced growth (**Fig. 1**) (*2*). IGF1-induced phosphorylation of Akt and MAPK in DU-145 cells was unaffected by 125B11, suggesting that 125B11 inhibits the cell-proliferative function of IGF1 in a way independent of the known IGF1-signaling pathway. In the second year of funding, we focused on analyzing the mechanism of action of 125B11.

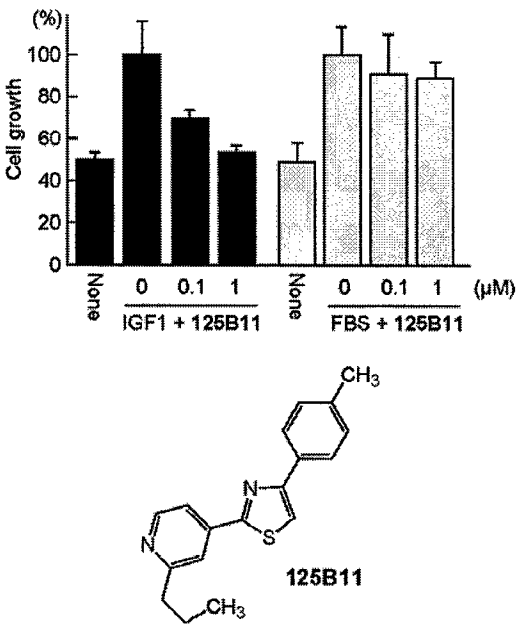

Fig. 1 Discovery of 125B11. 125B11 inhibited the IGF1-induced growth but not the serum-induced growth. DU-145 cells were treated with varied amounts of 125B11 in the presence of IGF1 or 2% fetal bovine serum (FBS).

Microarray experiments of 125B11

Gene expression profiling comparison in the drug-treated and -untreated cells by DNA microarray technology might reveal specific molecular pathways affected by the drugs. DU145 prostate cancer cells were treated with 125B11 or DMSO alone in the presence of IGF1, and extracted mRNA was analyzed by Affimetrix DNA micoarrays at the Microarray Facility in Baylor College of Medicine. To our surprise, we found that the genes downregulated by 125B11 included those known or likely to be controlled by sterol regulatory element binding protein (SREBP), a transcription factor that activates specific genes involved in cholesterol synthesis, endocytosis of low density lipoproteins, and the synthesis of both saturated and unsaturated fatty acids (*3*). These include LDL receptor and HMG-CoA reductase, a target of cholesterol-lowering drug statins. We hypothesized that 125B11, whether directly or indirectly, inhibits SREBP or its pathway.

Table 1

Microarray analysis of 125B11

Genes known to be controlled by SREBP

Genes relevant to sterol/fat synthesis

Downregulated by 125B11 in the presence of IGF1

0.615572	Hs.213289	NM_000527.2	low density lipoprotein receptor (familial hypercholesterolemia) /FL=gb:NM_000527.2
0.615572	Hs.75105	NM_006579.1	emopamil-binding protein (sterol isomerase) /FL=gb:NM_006579.1
0.615572	Hs.74304	NM_002705.1	Homo sapiens periplakin (PPL)
0.615572	Hs.268515	NM_002430.1	meningioma (disrupted in balanced translocation) 1 /FL=gb:NM_002430.1
0.615572	UG=Hs.311	AF096304.1	Homo sapiens putative sterol reductase SR-1 (TM7SF2) transmembrane 7 superfamily member 2 /F
0.615572	Hs.93199	D63807.1	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase) /FL=gb:D63807.1
0.615572	Hs.293007	BG153399	aminopeptidase puromycin sensitive
0.615572	Hs.301959	NM_007198.1	proline synthetase co-transcribed (bacterial homolog) /FL=gb:NM_007198.1
0.615572	Hs.15106	AC007182	chromosome 14 open reading frame 1
0.615572		NM_030802.1	CEBP-induced protein /DB_XREF=gi:13540589 /FL=gb:NM_030802.1
0.574349	Hs.119597	AB032261.1	stearoyl-CoA desaturase (delta-9-desaturase) /FL=gb:AF097514.1 gb:NM_005063.1 gb:AB032261.1
0.574349	Hs.174140	AI971281	ATP citrate lyase /FL=gb:NM_001096.1
0.574349	Hs.171825	NM_003670.1	basic helix-loop-helix domain containing, class B, 2 /FL=gb:AB004066.1 gb:NM_003670.1
0.574349	Hs.79103	AW235051	cytochrome b5 outer mitochondrial membrane precursor /FL=gb:BC004373.1 gb:NM_030579.1
0.574349	Hs.11806 7	NM_001360.1	dehydrocholesterol reductase /FL=gb:BC000054.1 gb:AF034544.1 gb:AF067127.1 gb:AF096305.1 g
0.574349	Hs.3838	NM_006622.1	serum-inducible kinase /FL=gb:AF059617.1 gb:NM_006622.1 gb:AF223574.1
0.574349	Hs.213289	AI861942	low density lipoprotein receptor (familial hypercholesterolemia) /FL=gb:NM_000527.2
0.574349	Hs.93199	AW084510	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase) /FL=gb:NM_002340.1 gb:U22526.1
0.574349	Hs.5920	NM_005476.2	UDP-N-acetylglucosamine-2-epimeraseN-acetylmannosamine kinase
0.574349	Hs.1524	NM_003811.1	tumor necrosis factor (ligand) superfamily, member 9 /FL=gb:NM_003811.1 gb:U03398.1
0.574349	Hs.278544	BC000408.1	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase) /FL=gb:BC000408.1
0.574349	Hs.174140	U18197.1	ATP citrate lyase /FL=gb:U18197.1
0.574349	Hs.7232	BE855983	acetyl-Coenzyme A carboxylase alpha /FL=gb:NM_000664.1 gb:U18622.1
0.574349	Hs.85266	AA808063	integrin, beta 4
0.574349	Hs.270594	AK001419.1	FLVCR protein /FL=gb:AF118637.1 gb:NM_014053.1
0.574349	Hs.102497	BF511170	paxillin
0.574349	Hs.326035	AI459194	early growth response 1
0.535887	Hs.226213	NM_000786.1	cytochrome P450, 51 (lanosterol 14-alpha-demethylase) /FL=gb:U23942.1 gb:NM_000786.1 gb:D5
0.535887	Hs.268490	NM_000475.2	nuclear receptor subfamily 0, group B, member 1 /FL=gb:NM_000475.2
0.535887	Hs.48876	AA872727	farnesyl-diphosphate farnesyltransferase 1 /FL=gb:L06070.1 gb:L06105.1 gb:NM_004462.1
0.535887	Hs.65370	NM_006033.1	lipase, endothelial /FL=gb:AF118767.1 gb:NM_006033.1
0.535887	Hs.14779	AK000162.1	acetyl-CoA synthetase
0.535887	Hs.44499	U59479.1	pinin, desmosome associated protein /DEF=Human neutrophil protein mRNA, partial cds.
0.5	Hs.154654	AU144855	cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile)
0.5	Hs.11899	AL518627	3-hydroxy-3-methylglutaryl-Coenzyme A reductase /FL=gb:M11058.1 gb:NM_000859.1
0.5	Hs.2178	NM_003528.1	H2B histone family, member Q /FL=gb:NM_003528.1
0.5	Hs.130607	NM_000431.1	mevalonate kinase (mevalonic aciduria) /FL=gb:M88468.1 gb:NM_000431.1
0.5	Hs.284244	NM_002006.1	fibroblast growth factor 2 (basic) /FL=gb:M27968.1 gb:NM_002006.1
0.5	Hs.71465	AF098865.1	squalene epoxidase /FL=gb:D78130.1 gb:AF098865.1 gb:NM_003129.2
0.5	Hs.288031	D85181.1	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase)-like /FL=gb:D85181.1
0.5		L32662.1	prostaglandin E2 receptor /DB_XREF=gi:484163 /FL=gb:L32662.1
0.5	Hs.81412	D80010.1	lipin 1
0.466517	Hs.11899	NM_000859.1	3-hydroxy-3-methylglutaryl-Coenzyme A reductase /FL=gb:M11058.1 gb:NM_000859.1
0.466517	Hs.79440	NM_006547.1	IGF-II mRNA-binding protein 3 /FL=gb:U97188.1 gb:U76705.1 gb:AF117106.1 gb:NM_006547.1
0.466517	Hs.75318	AL565074	tubulin, alpha 1 (testis specific)
0.466517	Hs.226213	U40053	cytochrome P450, 51 (lanosterol 14-alpha-demethylase)
0.466517	Hs.187579	NM_016371.1	hydroxysteroid (17-beta) dehydrogenase 7 /FL=gb:AF098786.2 gb:NM_016371.1
0.435275	Hs.76038	BC005247.1	isopentenyl-diphosphate delta isomerase /FL=gb:BC005247.1
0.435275	Hs.57698	BC000245.1	NAD(P) dependent steroid dehydrogenase-like; H105e3 /FL=gb:BC000245.1 gb:U47105.2 gb:NM_01
0.406126	Hs.75746	NM_000693.1	aldehyde dehydrogenase 1 family, member A3 /FL=gb:NM_000693.1 gb:U07919.1
0.406126	Hs.1690	NM_005130.1	heparin-binding growth factor binding protein /FL=gb:BC003628.1 gb:M60047.1 gb:NM_005130.1
0.406126	Hs.272897	AF141347.1	Tubulin, alpha, brain-specific /FL=gb:AF141347.1 gb:NM_006009.1
0.353553	Hs.77910	NM_002130.1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) /FL=gb:BC000297.1 gb:L25798.1 gb:M
0.329877	Hs.56205	BE300521	insulin induced gene 1 /FL=gb:NM_005542.1
0.329877	Hs.154654	NM_000104.2	cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile) /FL=g
0.329877	Hs.3828	AI189359	mevalonate (diphospho) decarboxylase /FL=gb:U49260.1 gb:BC000011.1 gb:NM_002461.1
0.329877	Hs.239926	AV704962	sterol-C4-methyl oxidase-like /FL=gb:U60205.1 gb:U93162.1 gb:NM_006745.2
0.094732	Hs.41749	NM_006259.1	protein kinase, cGMP-dependent, type II /FL=gb:D70899.1 gb:NM_006259.1

125B11 blocks cleavage of SREBP in cells

Goldstein, Brown, and colleagues have demonstrated that in the presence of sterol, 125 kDa SREBP is located endoplasmic reticulum membranes. When cellular sterol concentrations were lowered, the amino terminal 68 kDa domain of SREBP is released from the membrane, entered the nucleus, and bound to SREs in the promoters of SREBP-target genes. In contrast, the cleavage of SREBPs was prevented when cells were loaded with cholesterol/oxysterols; the result was low nuclear levels of SREBP and low rates of transcription of SREBP target genes (**Fig. 2**) (*4*).

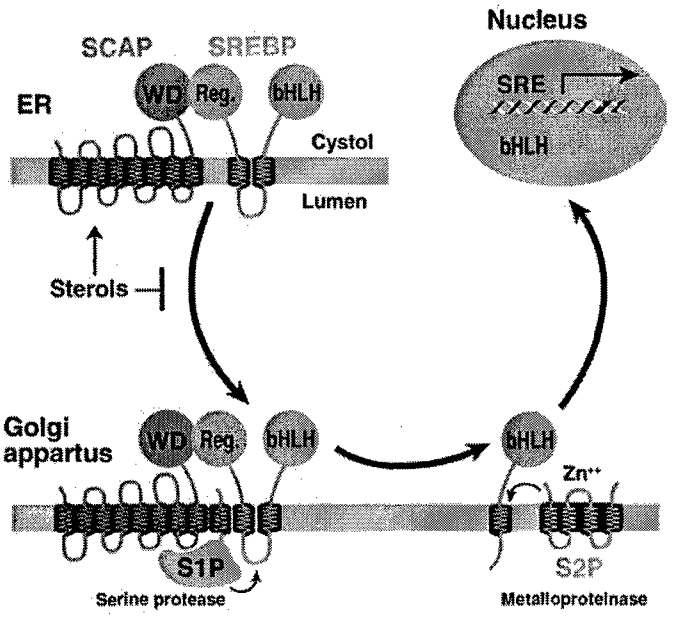

Fig. 2 Regulation of SREBP by sterols.

We examined if the cleavage of SREBP is blocked by 125B11 in cells. Western blot analysis showed that treatment with 5 μM of 125B11 increased the amounts of uncleaved 125Kda SREBP (**Fig. 3**).

It remains unknown how 125B11 inhibits the cleavage of SREBP. In sterol-deprived cells, a protein called SCAP functions as a chaperone protein and transports the 125 kDa SREBPs from the endoplasmic reticulum to the Golgi where two proteases (S1P and S2P) cleave SREBP to generate 68Kda SREBP (**Fig. 2**) (*3*). In contrast, in cholesterol-loaded cells, SCAP and SREBP fail to migrate to the Golgi and the 125 kDa SREBP remains in ER. Thus, under these conditions, maturation and nuclear localization of SREBPs are reduced. 125B11 may modulate one of these transport/cleavage process. Experiments are planned for the next year.

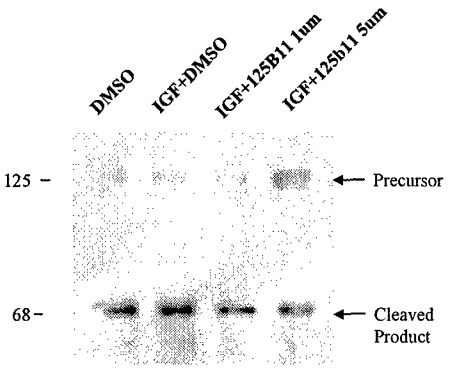

Fig. 3 Effect of 125B11 on SREBP-1 protein in DU145 cells. DU145 cells were seeded onto 6-well plates at a density of 2.5x10^5 with serum-free media for 16h before receive chemicals. Cells were then incubated for 6h with DMSO (line 1) or 1ug/ml IGF (lane 2) or IGF with 1uM 125B11 (lane 3) or IGF with 5uM 125B11 (lane 4) in non-serum medium. The levels of SREBP-1 in cell lysates were examined by Western blotting with an anti-SREBP-1 antibody.

SREBP plays a role in IGF1-dependent growth of prostate cancers?

Our results suggest that 125B11 suppress IGF1-dependent growth of prostate cancer cells by blocking SREBP activation. If this hypothesis is correct, then siRNA knockdown of SREBP suppress IGF1-dependent growth of prostate cancers. We stably transfected siRNA expression vector of SREBP to DU145 cells and examined its growth in the presence of IGF1. Although we need more validation experiments, the knowckdown cells seem to irresponsive to IGF1. This result may suggest that SREBP plays an essential role in IGF1-dependent growth and prostate cancer progression. Our continued experiments may reveal an interesting crosstalk between cholesterol metabolism and prostate cancers.

Key research accomplishments

- Identified SREBP-responsive genes as genes downregulated by 125B11
- Obtained evidence suggesting that 125B11 blocks the cleavage of SREBP
- Obtained preliminary results suggesting that SREBP plays an essential role in IGF1-dependent growth of prostate cancers.

Reportable outcomes

None during this funding period.
Results were discussed in 2004 Prostate Cancer Foundation Retreat

Conclusion

There is no change in Tasks and experimental design. As originally planned, discovery of 125B11 in the first year of funding enabled its mechanistic analysis in the second year. Our analysis indicated the potential role of SREBP in IGF1-dependent growth of prostate cancers. Continued experiments may reveal a molecular understanding of the relationship between cholesterol metabolism and prostate cancer progression. We are also interested in understanding how 125B11 blocks the cleavage of SREBP. Such studies may lead to the discovery of a new drug target.

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Appendix

None